

## Fungal degradation of paddy straw for enhancing biogas production

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### Abstract

Fungal mediated degradation of chopped and moist paddy straw (3-4cm) was investigated using *Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138 at different time intervals. The pre-treated paddy straw was in turn used for biogas production. Paddy straw pretreated with *T. reesei* MTCC 164 showed 20.8% enhanced biogas production and a maximum of 30% silica was removed after 25 days treatment. The paddy straw treated with *C. versicolor* MTCC 138 showed 26.2% enhanced biogas production with a maximum of 19.1 and 32.5% reduction in lignin and silica content respectively. Results regarding change in proximate and chemical composition of *T. reesei* MTCC 164 and *C. versicolor* MTCC 138 pretreated straw indicate that both these fungi might be the potential organisms for silica degradation.

**Keywords:** Paddy straw, biogas, agri-waste, fungal degradation, lignin, silica.

### Introduction

Paddy straw is one of the most abundant ligno-cellulosic wastes on the earth. In India, total annual production of rice was 136.5 million tons in the year 2009 (Anonymous, 2010). About 1-1.5 kg of straw is produced from every kilogram of the grain harvested (Maiorella, 1985) and thus, 136.5-150 million tons of paddy straw is estimated to be produced annually. In India, approximately 85-95 million tons of paddy straw is disposed off by burning. One ton of paddy straw burning releases 3 kg particulate matter, 60 kg CO, 1460 kg CO<sub>2</sub>, 199 kg ash and 2 kg SO<sub>2</sub> (Jenkins & Bhatnagar, 2003). Lung and respiratory diseases caused by burning adversely affect public health (Wang & Christopher, 2003). Repeated burning of paddy straw also results in soil erosion. The lignocellulosic biomass can be converted to biogas via anaerobic digestion (Kashyap *et al.*, 2003).

Anaerobic digestion is a biological process in which biodegradable organic material is decomposed in the absence of oxygen to produce biogas which is a mixture of CH<sub>4</sub> (55-75%), CO<sub>2</sub> (25-45%), H<sub>2</sub> (0-3%), N<sub>2</sub> (1-5%), CO (0-0.3%), H<sub>2</sub>S (0.1-0.5), O<sub>2</sub> and water vapors (traces) (Pauss *et al.*, 1987). The organic matter can be degraded by the sequential action of hydrolytic, acetogenic and methanogenic bacteria to produce biogas. Although, paddy straw has high cellulose content but the lignin complex and silica incrustation shields the microbial action for biogas production.

Therefore, the paddy straw needs to be pretreated to enable cellulose to be more accessible to the microbial/enzymatic attack. Many physical (mechanical and non-mechanical), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physico-chemical (Ammonia fibre explosion, CO<sub>2</sub> and steam explosion) and biological pretreatments (lignocellulolytic micro-organisms and the enzymes) have

been proposed in the recent years (Saratale *et al.*, 2008; Hendriks & Zeeman, 2009). However, the physical and chemical pretreatments require high energy and corrosion resistant, high-pressure reactors, which increase the need of equipment and cost of pretreatment. Furthermore, the chemical pretreatments can be detrimental to the methanogens apart from generating acidic or alkaline water, which needs pre-disposal treatment to ensure environment safety (Keller *et al.*, 2003).

Thus, an alternative approach is microbial pretreatment especially fungi to increase digestibility of paddy straw. Advantages of biological pretreatment include inexpensive, low energy requirement and mild environmental conditions (Saratale *et al.* 2008). Most of the white-rot fungi degrade lignin and cellulose simultaneously. A selective white-rot fungus, *Ceriporiopsis subvermispora* is known to selectively degrade lignin in softwood and hardwood (Okano *et al.*, 2005). No doubt, reports are available for biological pretreatment of paddy straw; however, the effect of pretreatment on biogas production is virtually nil.

In the present study, the chopped and moist paddy straw was pretreated with, *Trichoderma reesei* MTCC 164 (cellulolytic fungus) and *Coriolus versicolor* MTCC 138 (ligno-cellulolytic fungus) and their effect on change in chemical composition of paddy straw was determined. The correlation between change in various contents of paddy straw and biogas production was developed.

### Materials and methods

Fresh paddy straw (*Oryza sativa* L.) was procured from the research field of Punjab Agricultural University, Ludhiana after harvesting the crop in the month of October and November. The paddy straw was chopped to 3-4 cm size with a Toka machine and was stored in polythene bags at room temperature. All the chemicals

Table 1. Growth characteristics of standard microbial cultures

Culture	Characteristic feature	Media used	Incubation temperature	Incubation period (days)
<i>Trichoderma reesei</i> MTCC 164	Cellulolytic	Malt extract agar (MEA)	25°C±2	7-8
<i>Coriolus versicolor</i> MTCC 138	Ligno-cellulolytic	Glucose yeast extract (GYE)	27°C±2	8-10

used for media, solutions preparation and proximate analysis were of analytical grade and were purchased from Hi-Media, Loba-Chemie and S.D. fine chemicals Pvt. Ltd. Cattle dung was procured from Dairy farm, GADVASU (Guru Angad Dev Veterinary & Animal Science University), Ludhiana. Digested cattle dung slurry was procured from a working biogas plant in biogas field laboratory of School of Energy Studies for Agriculture, PAU, Ludhiana and was used as inoculum for biogas production

Active standard fungal cultures were procured from MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh. The cultures were maintained as per the growth conditions given in Table 1 and were stored in refrigerator after sub-culturing.

Table 2. Change in chemical and proximate composition of paddy straw pretreated with *Trichoderma reesei* MTCC 164

Composition	Pretreatment period days (% change)						CD (5%)
	Control <sup>#</sup>	5	10	15	20	25	
Total solids (TS %)	22.0±0.12	20.9±0.15 (5.0↓)	19.8±0.08 (10.0↓)	19.5±0.16 (11.0↓)	19.0±0.09 (14.0↓)	18.0±0.14 (18.0↓)	0.72
Volatile solids (VS %)	83.8±0.06	83.0±0.09 (0.96↓)	81.7±0.05 (2.5↓)	80.6±0.06 (3.8↓)	79.1±0.07 (5.6↓)	78.3±0.08 (6.5↓)	0.19
Ash (%)	16.2±0.06	17.0±0.05 (5.0↑)	18.3±0.09 (13.0↑)	19.4±0.05 (17.0↑)	20.9±0.07 (28.0↑)	21.7±0.08 (34.0↑)	0.22
Total organic carbon (%)	46.5±0.16	46.1±0.11 (0.8↓)	45.4±0.20 (2.4↓)	44.7±0.12 (3.9↓)	43.9±0.11 (5.6↓)	43.5±0.13 (6.5↓)	0.19
Cellulose (%)	34.4±0.14	34.3±0.16 (0.3↓)	34.2±0.12 (0.6↓)	30.4±0.23 (12.8↓)	29.5±0.23 (14.2↓)	28.9±0.19 (15.9↓)	0.56
Hemicellulose (%)	23.8±0.22	22.1±0.19 (7.2↓)	19.0±0.16 (20.5↓)	20.6±0.32 (13.5↓)	24.0±0.21 (0.85↑)	24.8±0.32 (5.2↑)	0.16
Lignin (%)	8.2±0.12	8.9±0.08 (8.5↑)	9.8±0.08 (19.5↑)	10.2±0.11 (24.0↑)	10.8±0.09 (31.7↑)	11.6±0.06 (41.4↑)	0.19
Silica (%)	11.0±0.05	10.1±0.06 (8.2↓)	8.5±0.08 (22.7↓)	8.2±0.08 (25.5↓)	7.9±0.09 (28.2↓)	7.7±0.11 (30.0↓)	0.17
Total sugar (mg/g PS)	43.0±0.07	45.1±0.05 (4.7↑)	49.1±0.06 (13.9↑)	52.3±0.08 (20.9↑)	58.2±0.06 (34.8↑)	63.3±0.05 (31.7↑)	0.22
TS(g/kg PS)	220.0±0.17	209.0±0.13 (5.0↓)	195.0±0.12 (11.4↓)	192.0±0.16 (12.7↓)	190.0±0.19 (13.6↓)	180.0±0.13 (18.2↓)	0.14
VS(g/kg PS)	184.4±0.11	173.5±0.09 (5.9↓)	157.2±0.14 (14.8↓)	156.2±0.16 (15.3↓)	150.1±0.23 (18.6↓)	140.9±0.19 (23.6↓)	0.95

<sup>#</sup>Control: Untreated paddy straw; PS: Paddy straw; CD: Critical difference; ± values indicate % Standard error for triplicate data; ↓: decrease; ↑: increase

### Pretreatment of paddy straw

Two standard cultures namely, *Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138 were used for biological pretreatment studies. The inoculum required for pretreatment of paddy straw was prepared on wheat grains. The grains were washed and boiled for 20-30

minutes till tender. The excess water was drained off. The grains were then mixed with 2 % gypsum (CaSO<sub>4</sub>) and 4% calcium carbonate (CaCO<sub>3</sub>). The grains were dispensed into empty glucose bottles (250 g/bottle) or autoclavable poly bags. The bottles were cotton plugged and autoclaved for 90 minutes. The poly bags were plugged by using plastic ring which supported the cotton plug. After cooling the bottles or poly bags were inoculated by placing two bits (1cm) of 7-8 days old culture on the opposite sides of bottle. The bottles/poly bags were incubated at 30±2°C, till the mycelium completely impregnated the wheat grains (8-10 days).

The paddy straw (250g) was chopped and soaked overnight. The excess water was drained off. The paddy straw was then inoculated with inoculum (fungus impregnated wheat grains) at the rate of 10% w/w (25g in 250g paddy straw). All these sets were incubated at required temperature for different durations of time i.e. 5,

10, 15, 20 and 25 days. After the completion of incubation period, pretreated paddy straw was dried at 100 C after washing. The pretreated straw was kept in polythene bags and was used for determining the change in composition. All the experiments for proximate analysis were conducted in triplicates.

## Biogas production

Biogas production experiments were carried out in two litre capacity digesters following monophasic method and biogas produced was measured by water displacement method. Two hundred and fifty gram pretreated moist paddy straw was mixed with 250g digested cattle dung slurry and 100g cattle dung. The mixture was filled in biogas digester. The digested cattle dung slurry act as inoculum for biogas production whereas cattle dung act as inducer for enhancing biogas production from paddy straw. The digester was properly sealed with rubber cork and araldite. This was connected to water displacement system for measuring biogas production.

Standard methods of AOAC (2000) were followed for the determination of total solids (TS), volatile solids (VS), ash, cellulose, hemi-cellulose, lignin and silica. Total sugars were estimated by phenol-sulphuric acid method using glucose as standard (Dubois *et al.*, 1956). All treatments were completed in triplicate. Critical difference at 5% level was performed for both chemical analysis and biogas data using Completely Randomized Designs (CRD) program in the CPCS1 software developed by Department of Statistics, PAU, Ludhiana. Standard error was calculated manually for all the experiments.

## Results and discussion

### Effect of *T. reesei* MTCC 164 on paddy straw degradation and biogas production

Results from Table 2 indicate that there was a gradual and significant decrease in total solids (TS) and volatile solids (VS) with increase in incubation period. The TS and VS decreased from 22% (in control) to 18% and from 83.8% (in control) to 78.3% in 25 days treatment, respectively. The cellulose content remained more or less constant till 10 days, however, further incubation led to decrease in cellulose content by 15.9%. Initially a decreasing trend of hemicellulose (HC) content was observed for a period of 10 days. However, further increase in pretreatment period led to increase in hemicellulose content. Initial decrease in hemicellulose content for first ten days might be the result of breakdown or hydrolysis of hemicellulose into fermentable sugars (Jalc *et al.*, 1998). This observation clearly indicates that the fungus has active hemicellulases during 10 days of its growth cycle and active cellulases after 10 days period. Initially hemicellulose and then cellulose was utilized for growth and multiplication of fungus (Mehta *et al.*, 1990). However, further

increase in pretreatment period led to increase in hemicellulose content as some inhibitors are formed like furfurals and hydroxyl-methyl furfurals. Thus *T. reesei* can only convert the hexoses, such as glucose and mannose, and not the pentoses, such as xylose and arabinose, that are found in the hemicellulose part of the straw (Klinke *et al.*, 2003, Petersson *et al.*, 2007). Hence, 10 days of incubation period was sufficient to increase the digestibility of paddy straw. Lignin content increased with the increase of incubation period reaching 11.6% in 25 days treated sample, as *T. reesei* is a cellulolytic fungus. Decrease in silica was smooth and significant up to 10 days of incubation and then almost remain stable up to incubation of 20 days. A maximum of 30% silica was removed after 25 days treatment. Reports are available showing that silica solubilizing microbes are available in nature (Ehrlich, 2006). Therefore, *T. reesei* could be a potential organism for silica solubilization.

Similar results were also observed by Jalc *et al.* (1998), who reported maximum loss in hemicellulose content in white rot fungus treated wheat straw. *Pleurotus ostreatus* has been found to be the most promising fungus in increasing cellulose content and decreasing Klason lignin of rice straw (Taniguchi *et al.*, 2005). However, Kirk and Farrell (1987) observed that 18 *Trichoderma* strains and *Trichoderma harzianum* were found to degrade lignin significantly.

Results from Table 3 indicate that biogas production increased in 5 and 10 days pretreated paddy straw. A maximum of 20.8% biogas production was observed in 10 days pretreated straw. Similar results also reported by Petersson *et al.* (2007). However, further increase in pretreatment time led to a decrease of biogas production as 25 days pretreated straw reflected 50.9% decrease in biogas production compared to control. The increase in biogas production might be due to the increase in digestibility of paddy straw by decrease in silica content and breakage of bonds between cellulose, hemicellulose and lignin content (Fox & Noike, 2004). Further reduction in biogas production can be correlated with the decrease in substrate, preferably cellulose, which is preferable source for methanogens and hinderance caused by lignin barrier. Lignin degradation is primarily an aerobic process

Table 3. Biogas production profile of *Trichoderma reesei* MTCC 164 pretreated paddy straw

Biogas parameters	Pretreatment period (days)					
	Control <sup>#</sup>	5	10	15	20	25
l/250g PS	32.2±9.4	34.8±8.9	38.9±10.6	37.2±11.2	23.5±12.3	15.8±7.9
l/kg PS	124.8±12.8	139.2±18.5	155.5±24.5	148.8±20.8	94.0±24.2	63.2±20.8
l/kg TS*	581.8±22.5	666.0±13.3	797.9±24.8	775.0±18.5	494.7±19.2	351.1±21.0
l/kg VS*	698.8±14.6	802.3±17.1	989.8±20.8	952.6±25.6	626.6±19.8	448.5±22.8
% change from control	0.0	8.1 (↑)	20.8 (↑)	15.5 (↑)	27.1 (↓)	50.9 (↓)

<sup>#</sup>Control: Untreated paddy straw; PS: Paddy straw; \*The values of biogas l/kg TS and biogas l/kg VS taken from Table 2; Average of triplicate data; Composition of paddy straw mixture: 250g paddy straw +250g digested cattle dung slurry +100g cattle dung; Biogas digester: 2 litre capacity; incubation temperature: 37±2°C; incubation period: 35 days; The amount of biogas produced from cattle dung & slurry was deducted from the amount of biogas produced from paddy straw+slurry+cattle dung.

Table 4. Change in chemical and proximate composition of *Coriolus versicolor* MTCC 138 pretreated paddy straw

Composition	Pretreatment period days (% change)						CD (5%)
	Control <sup>#</sup>	5 days	10	15	20	25	
Total solids (TS%)	29.5±0.12	28.3±0.13 (4.1↓)	24.9±0.15 (15.6↓)	23.3±0.12 (21.1↓)	22.4±0.12 (24.1↓)	21.2±0.18 (28.2↓)	0.26
Volatile solids (VS%)	82.5±0.05	80.5±0.07 (2.4↓)	77.8±0.08 (5.7↓)	76.1±0.06 (7.8↓)	75.9±0.06 (8.0↓)	75.2±0.06 (8.8↓)	0.23
Ash(%)	17.6±0.08	19.5±0.09 (10.8↑)	22.2±0.08 (26.1↑)	23.8±0.11 (35.2↑)	24.1±0.07 (36.9↑)	24.8±0.08 (40.9↑)	0.17
Total organic carbon (%)	45.8±0.18	44.7±0.21 (2.4↓)	43.2±0.19 (5.7↓)	42.3±0.19 (7.6↓)	42.2±0.22 (7.9↓)	41.8±0.14 (8.7↓)	0.19
Cellulose (%)	32.2±0.12	31.0±0.11 (3.7↓)	30.2±0.14 (6.2↓)	28.0±0.09 (13.1↓)	26.8±0.15 (16.8↓)	26.0±0.11 (19.3↓)	0.28
Hemicellulose (%)	22.2±0.14	20.8±0.13 (6.3↓)	21.2±0.13 (4.5↓)	22.0±0.18 (0.9↓)	22.8±0.21 (2.7↑)	23.6±0.17 (6.3↑)	0.26
Lignin (%)	12.0±0.08	11.1±0.08 (7.5↓)	10.9±0.09 (9.2↓)	10.5±0.08 (12.5↓)	9.9±0.08 (17.5↓)	9.1±0.06 (19.1↓)	0.17
Silica (%)	12.0±0.11	10.5±0.17 (12.5↓)	9.8±0.12 (18.4↓)	9.3±0.15 (22.5↓)	8.9±0.17 (25.8↓)	8.1±0.11 (32.5↓)	0.20
Total sugar (mg/ g PS)	43.0±0.07	65.1±0.08 (53.4↑)	85.2±0.09 (97.6↑)	90.0±0.07 (109.3↑)	92.2±0.06 (113.9↑)	96.6±0.05 (123.2↑)	0.19
TS (g/kg PS)	294.6±0.14	283.2±0.15 (3.9↓)	249.7±0.14 (15.2↓)	232.6±0.14 (21.1↓)	223.3±0.17 (24.2↓)	211.1±0.14 (28.3↓)	0.25
VS (g/kg PS)	242.8±0.12	228.0±0.17 (6.1↓)	194.2±0.19 (20.1↓)	177.1±0.16 (27.1↓)	169.6±0.16 (30.1↓)	158.7±0.11 (34.6↓)	0.43

<sup>#</sup>Control: Untreated paddy straw; PS: Paddy straw; CD: Critical difference; ↓: decrease; ↑: increase; ± values indicate % Standard error for triplicate data

and in an anaerobic environment lignin can persist for very long periods (Van Soest, 2006). Because lignin is the most recalcitrant component of the plant cell wall, the higher the proportion of lignin the lower the bioavailability of the substrate. The effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity (Haug, 1993).

#### Effect of *C. versicolor* MTCC 138 on paddy straw degradation and biogas production:

Results from Table 4 indicate that there was a gradual and significant decrease in total solids (TS) and volatile solids (VS) with increase in incubation period. The TS and VS decreased from 29.5% (in control) to 21.2% and 82.5% (in control) to 75.2% in 25 days treatment, respectively. The cellulose content kept on decreasing with the increasing incubation period, with the maximum reduction of 19.3% after 25 days pretreatment. Initially a decreasing trend of hemicellulose content was observed for a period of 10 days, however further increase in pretreatment period led to increase in hemicellulose content by 6.3%. Initial decrease in hemicellulose content for first ten days might be the result of breakdown or hydrolysis of hemicellulose into fermentable sugars (Jal

et al., 1998). This observation clearly indicates that the fungus has active hemicellulases during initial phase of its growth cycle and active cellulases after 10 days period. Mehta et al (1990) too reported that initially hemicellulose and then cellulose was utilized for growth and multiplication of fungus. However, further increase led to biosynthesis of hemicellulose from the byproducts of cellulose, which showed decreasing trend continuously. Lignin content decreased with the increase of incubation period with maximum reduction of 19.1% in 25 days treated sample. Decrease in silica was smooth and significant with a maximum removal of 32.5%. The decreased lignin and silica content of paddy straw are responsible for increased digestibility as these are the main inderance in paddy straw utilization. Reports are also available showing that silica solubilizing microbes are available in nature (Ehrlich 2006). Therefore, *C. versicolor* was found to be a potential organism for silica and lignin degradation/utilization.

Results from Table 5 indicate that enhanced biogas production was found in 5, 10 and 15 days pretreated paddy straw, however, maximum biogas production was observed in 5 days pretreated straw which showed 26.2% increase over control. However, further increase in pretreatment time after 15 days led to a decrease in biogas production. Twenty five days pretreated straw

Table 5. Biogas production profile of *Coriolus versicolor* MTCC 138 pretreated paddy straw

Biogas parameters	Pretreatment period (days)					
	Control <sup>#</sup>	5	10	15	20	25
l/250g PS	33.6±19.4	42.4±11.2	37.6±11.5	34.9±8.9	32.8±9.7	31.2±7.5
l/kg PS	134.4±11.3	169.6±13.9	150.4±15.6	139.6±18.1	131.2±19.8	124.8±21.5
l/kg TS*	454.9±23.1	598.9±15.6	587.6±16.7	599.6±18.0	588.4±18.9	591.2±21.0
l/kg VS*	551.9 ±15.6	743.9±18.6	755.5±21.6	787.5±25.8	774.8±19.0	786.0±23.2
% change from control	0.0	26.2 (↑)	11.9 (↑)	3.8 (↑)	2.3 (↓)	7.1 (↓)

<sup>#</sup>Control: Untreated paddy straw; PS: Paddy straw; \*The values of biogas l/kg TS and biogas l/kg VS taken from Table 4; Average of triplicate data; Composition of paddy straw mixture: 250g paddy straw +250g digested cattle dung slurry +100g cattle dung; Biogas digester: 2 litre capacity; incubation temperature: 37±2°C; incubation period: 35 days; The amount of biogas produced from cattle dung & slurry was deducted from the amount of biogas produced from paddy straw + cattle dung + slurry

resulted in 6.9% reduction in biogas as compared to control. The increase in biogas production might be due to the increase in digestibility of paddy straw by decrease in silica content and breakage of bonds between cellulose, hemicellulose and lignin content (Fox & Noike, 2004). Further reduction in biogas production can be correlated with the decrease in substrate, preferably cellulose, which is preferable source for methanogens and hinderance caused by lignin barrier. Lignin degradation is primarily an aerobic process and in an anaerobic environment lignin can persist for very long periods (Van Soest, 2006). Because lignin is the most recalcitrant component of the plant cell wall, the higher the proportion of lignin the lower the bioavailability of the substrate. The effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity (Haug, 1993).

Similar results were also observed by Jafari *et al.* (2007), where decrease in hemicellulose, acid detergent fibre and neutral detergent fibre was observed after pretreatment of rice straw with *Pleurotus spp.* Zafar *et al.* (1980) also showed that there was significant reduction in cellulose content after treatment of rice straw by *Pleurotus sajor caju*. The increase in biogas production during initial treatment period might be due to the increase in digestibility of paddy straw by decrease in silica and lignin content and breakage of bonds between cellulose, hemicellulose and lignin content. Further decrease in biogas production can be correlated with the decrease in cellulose and hemicellulose content of paddy straw with increasing incubation period as cellulose is preferred substrate for methanogens for biogas production.

## Conclusion

*Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138 enhance biogas production from paddy straw by 20.8 and 26.2% respectively. As reports about presence of silica solubilizing microbes in nature are available (Ehrlich, 2006), therefore *T. reesei* MTCC 164 and *C. versicolor* MTCC 138 might be the potential organisms for silica degradation/utilization.

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